PATENT COOPERATION TREATY

From the
INTERNATIONAL SEARCHING AUTHORITY

To:
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PCT

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

(PCT Rule 43*bis*.1)

Date of mailing *(day/month/year)*	17 FEB 2006
Applicant's or agent's file reference 27558	**FOR FURTHER ACTION** See paragraph 2 below

International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/IL04/00181	24 February 2004 (24.02.2004)	27 April 2003 (27.04.2003)

International Patent Classification (IPC) or both national classification and IPC

IPC(8): C12P 21/06; C12N 9/00, 9/14, 1/12, 1/20, 5/00, 15/00; C07H 21/04; A01H 11/00 and US Cl.: 435/4, 6, 41, 69.1, 183, 195, 252.1, 252.3, 254.1, 320.1, 325, 410,; 536/23.1, 23.4, 23.5; 800/295

Applicant

METABOGAL, LTD

1. This opinion contains indications relating to the following items:

- ☒ Box No. I — Basis of the opinion
- ☐ Box No. II — Priority
- ☐ Box No. III — Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
- ☒ Box No. IV — Lack of unity of invention
- ☒ Box No. V — Reasoned statement under Rule 43*bis*.1(a)(i) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
- ☐ Box No. VI — Certain documents cited
- ☐ Box No. VII — Certain defects in the international application
- ☐ Box No. VIII — Certain observations on the international application

2. **FURTHER ACTION**

If a demand for international preliminary examination is made, this opinion will be considered to be a written opinion of the International Preliminary Examining Authority ("IPEA") except that this does not apply where the applicant chooses an Authority other than this one to be the IPEA and the chosen IPEA has notified the International Bureau under Rule 66.1*bis(b)* that written opinions of this International Searching Authority will not be so considered.

If this opinion is, as provided above, considered to be a written opinion of the IPEA, the applicant is invited to submit to the IPEA a written reply together, where appropriate, with amendments, before the expiration of 3 months from the date of mailing of Form PCT/ISA/220 or before the expiration of 22 months from the priority date, whichever expires later.

For further options, see Form PCT/ISA/220.

3. For further details, see notes to Form PCT/ISA/220.

Name and mailing address of the ISA/ US	Date of completion of this opinion	Authorized officer
Mail Stop PCT, Attn: ISA/US Commissioner for Patents P.O. Box 1450 Alexandria, Virginia 22313-1450 Facsimile No. (571) 273-3201	15 November 2005 (15.11.2005)	Manjunath N. Rao, Ph.D. Telephone No. 571-272-1600

Form PCT/ISA/237 (cover sheet) (April 2005)

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

International application No.
PCT/IL04/00181

Box No. I Basis of this opinion

1. With regard to the **language**, this opinion has been established on the basis of:

☒ the international application in the language in which it was filed

☐ a translation of the international application into _____, which is the language of a translation furnished for the purposes of international search (Rules 12.3(a) and 23.1(b)).

2. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application and necessary to the claimed invention, this opinion has been established on the basis of:

a. type of material

☒ a sequence listing

☐ table(s) related to the sequence listing

b. format of material

☒ on paper

☒ in electronic form

c. time of filing/furnishing

☒ contained in the international application as filed.

☒ filed together with the international application in electronic form.

☐ furnished subsequently to this Authority for the purposes of search.

3. ☐ In addition, in the case that more than one version or copy of a sequence listing and/or table(s) relating thereto has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

4. Additional comments:

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Box No. IV Lack of unity of invention

1. ☒ In response to the invitation (Form PCT/ISA/206) to pay additional fees the applicant has, within the applicable time limit:
   - ☒ paid additional fees
   - ☐ paid additional fees under protest and, where applicable, the protest fee
   - ☐ paid additional fees under protest but the applicable protest fee was not paid
   - ☐ not paid additional fees
2. ☐ This Authority found that the requirement of unity of invention is not complied with and chose not to invite the applicant to pay additional fees.
3. This Authority considers that the requirement of unity of invention in accordance with Rule 13.1, 13.2 and 13.3 is
   - ☐ complied with
   - ☒ not complied with for the following reasons:

   See the lack of unity section of the International Search Report(Form PCT/ISA/210)

4. Consequently, this opinion has been established in respect of the following parts of the international application:
   - ☐ all parts.
   - ☒ the parts relating to claims Nos. 1-24,28-31,33-37 and 42

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

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Box No. V Reasoned statement under Rule 43 *bis*.1(a)(i) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Claims 4, 12-24, 42	YES
	Claims 1-3, 5-11, 28-31, 33-37	NO
Inventive step (IS)	Claims NONE	YES
	Claims 1-24, 28-31, 33-37, 42	NO
Industrial applicability (IA)	Claims 1-24, 28-31, 33-37, 42	YES
	Claims NONE	NO

2. Citations and explanations:

Please See Continuation Sheet

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Supplemental Box
In case **the space in any of the preceding boxes is not sufficient.**

V. 2. Citations and Explanations:
Claims 1-3, 5-11, 28-31, 33-37 lack novelty under PCT Article 33(2) as being anticipated by Martin et al. (DNA, 1988, Vol. 7, No.2, pages 99-106). Claims 1-3, 5-11, 28-31, 33-37 are drawn to a host cell producing a high mannose recombinant protein comprising a polynucleotide encoding the recombinant protein and a signal for causing the recombinant protein to be produced as a high mannose protein, wherein the polynucleotide comprises a first nucleic acid sequence encoding said protein of interest operably linked to a second nucleic acid sequence encoding a signal peptide wherein said signal peptide comprises a ER targeting peptide and wherein said host cell is a prokaryotic or a eukaryotic host cell and wherein said polypeptide is one of the lysosomal proteins such as glucocerebrosidase. Claim 28-31, 33-37 are also drawn to a recombinant biologically active high mannose lysosomal enzyme having at least one oligosaccharide chain comprising an exposed mannose residue. Martin et al. disclose one such host cell comprising a polynucleotide encoding said enzyme wherein said polypeptide is produced as a high-mannose protein in high levels. Matin et al. also disclose a recombinant glucocerebrosidase wherein said enzyme is inherently a biologically active high mannose lysosomal enzyme having at least one oligosaccharide chain comprising an exposed mannose residue. Thus, Martin et al. anticipate claims 1-3, 5-11, 28-31, 33-37 as written.

Claims 4, 12-24 and 42 lack an inventive step under PCT Article 33(3) as being obvious over the prior art as applied in the immediately preceding paragraph and further in view of Boller et al. and Zhu et al. Claims 4, 12-24 and 42 are drawn to a host cell producing a high mannose recombinant protein comprising a polynucleotide encoding the recombinant protein and a signal for causing the recombinant protein to be produced as a high mannose protein, wherein the polynucleotide comprises a first nucleic acid sequence encoding said protein of interest operably linked to a second nucleic acid sequence with with SEQ ID NO:1 encoding a signal peptide wherein said signal peptide comprises a ER targeting peptide and wherein said polynucleotide is operably linked to a third polynucleotide sequence with SEQ ID NO:2 encoding a plant vacuolar targeting sequence, and wherein said host cell is a plant cell and wherein said polypeptide is one of the lysosomal proteins such as glucocerebrosidase. Claim 42 is drawn to a recombinant protein produced from a plant host cell. The reference of Martin et al. has already been discussed above. Martin et al. teach the production of glucocerebrosidase, a lysosomal protien recombinantly using a host cell comprising a polynucleotide with a signal sequence. The reference of Zhu et al. teach the polynucleotide encoding the signal peptide SEQ ID NO:1 and its use in producing novel recombinant proteins. On similar lines Boller et al. teach the vacuolar targeting sequence SEQ ID NO:2 and its use in targeting polypeptides into the vacuolar space. The invention as a whole is directed to production of glucocerebrosidase as a transgenic protein in plant host cells. The art and the above references teach and provide all sequences required for expressing the glucocerebrosidase as a transgenic protein. The production of mammalian proteins in plant products such as fruits and seed is well known since it eliminates the steps of purification and makes the recombinant protein ready for administration as a plant product. Therefore, with the above references in hand, it would have been obvious to one of ordinary skill in the art to produce human glucocerebrosidase, which is used in enzyme replacement therapy for lysosomal enzyme disorders, as a plant protein by expressing as a polynucleotide linked to the above signal sequence and vacuolar targeting sequences. One of ordinary skill in the art would have been motivated to do so since the lysosomal protein is extensively used in enzyme replacement therapy and production of the protein as a plant product would avoid the extensive purification steps and can be easily administered as a plant

Supplemental Box
In case **the space in any of the preceding boxes is not sufficient.**

product. One of ordinary skill in the art would have had a reasonable expectation of success since Martin et al. already provide a host cell producing the high-mannose protein, Zhu et al. and Boller et al. provide the sequences to make a DNA construct to be expressed in a plant cell. Therefore the above invention would have been *prima facie* obvious to one of ordinary skill in the art.

Claims 1-24, 28-31, 33-37, 42 meet the criteria set out in PCT Article 33(4), and thus have industrial applicability because the subject matter claimed can be made or used in industry.

NOTES TO FORM PCT/ISA/220

These Notes are intended to give the basic instructions concerning the filing of amendments under Article 19. The Notes are based on the requirements of the Patent Cooperation Treaty, the Regulations and the Administrative Instructions under that Treaty. In case of discrepancy between these Notes and those requirements, the latter are applicable. For more detailed information, see also the *PCT Applicant's Guide*, a publication of WIPO.

In these Notes, "Article," "Rule" and "Section" refer to the provisions of the PCT, the PCT Regulations and the PCT Administrative Instructions, respectively.

INSTRUCTIONS CONCERNING AMENDMENTS UNDER ARTICLE 19

The applicant has, after having received the international search report, one opportunity to amend the claims of the international application. It should however be emphasized that, since all parts of the international application (claims, description and drawings) may be amended during the international preliminary examination procedure, there is usually no need to file amendments of the claims under Article 19 except where, e.g. the applicant wants the latter to be published for the purposes of provisional protection or has another reason for amending the claims before international publication. Furthermore, it should be emphasized that provisional protection is available in some States only.

What parts of the international application may be amended ?

Under Article 19, only the claims may be amended.

During the international phase, the claims may also be amended (or further amended) under Article 34 before the International Preliminary Examining Authority. The description and drawings may only be amended under Article 34 before the International Preliminary Examining Authority.

Upon entry into the national phase, all parts of the international application may be amended under Article 28 or, where applicable, Article 41.

When ? Within 2 months from the date of transmittal of the international search report or 16 months from the priority date, whichever time limit expires later. It should be noted, however, that the amendments will be considered as having been received on time if they are received by the International Bureau after the expiration of the applicable time limit but before the completion of the technical preparations for international publication (Rule 46.1).

Where not to file the amendments ?

The amendments may only be filed with the International Bureau and not with the receiving Office or the International Searching Authority (Rule 46.2).

Where a demand for international preliminary examination has been/is filed, see below.

How ? Either by cancelling one or more entire claims, by adding one or more new claims or by amending the text of one or more of the claims as filed.

A replacement sheet must be submitted for each sheet of the claims which, on account of an amendment or amendments, differs from the sheet originally filed.

All the claims appearing on a replacement sheet must be numbered in Arabic numerals. Where a claim is cancelled, no renumbering of the other claims is required. In all cases where claims are renumbered, they must be renumbered consecutively (Administrative Instructions, Section 205(b)).

The amendments must be made in the language in which the international application is to be published.

What documents must/may accompany the amendments ?

Letter (Section 205(b)):

The amendments must be submitted with a letter.

The letter will not be published with the international application and the amended claims. It should not be confused with the "Statement under Article 19(1)" (see below, under "Statement under Article 19(1)").

The letter must be in English or French, at the choice of the applicant. However, if the language of the international application is English, the letter must be in English; if the language of the international application is French, the letter must be in French.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US01/25882

BOX II. OBSERVATIONS WHERE UNITY OF INVENTION WAS LACKING
This ISA found multiple inventions as follows:

This application contains the following inventions or groups of inventions which are not so linked as to form a single inventive concept under PCT Rule 13.1. In order for all inventions to be searched, the appropriate additional search fees must be paid.

Group I, claim(s)1-80, drawn to a method of use of a high mannose glucocerebrosidase (hmGCB), a method of making hmGCB and a preparation, including the pharmaceutical one, comprising hmGCB.
Group II, claim(s) 81-104, drawn to methods of purifying hmGCB.

The inventions listed as Groups I and II do not relate to a single inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons: they are drawn to materially different methods. The invention of Group I is drawn to a method of use of hmGCB of any purity and to a method of making the same whereas the invention of Group II is drawn to a special technical feature of a method of purifying hmGCB, said method employing techniques different from the ones used in methods of invention I.

37 CFR 1.475 does not provide for multiple products or methods within a single application and therefore, unity of invention is lacking with regard to Groups I and II.